

Appl. No. 09/881,204  
Amendment Dated January 18, 2005  
Reply to Office Action of November 18, 2004

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

Claims 1-47 (canceled)

Claim 48 (currently amended) A method of generating a hybrid mammalian cell comprising:

- (a) preparing more than one cytoplasm fragment from a mammalian metaphase II oocyte or fertilized zygote wherein the amount of cytoplasm in the cytoplasm fragment is less than the amount of cytoplasm in the mammalian oocyte or fertilized zygote;
- (b) obtaining a nuclear donor cell or karyoplast taken from a mammal;
- (c) combining a cytoplasm fragment of step a) with the nuclear donor cell or karyoplast of step b) to produce a hybrid mammalian cell; and
- (d) if an oocyte is used in step (a), then activating the oocyte before, during or after step (c).

Claim 49 (previously presented) The method of claim 48, wherein the cytoplasm fragment is produced by vortexing the mammalian oocyte or fertilized zygote.

Claim 50 (previously presented) The method of claim 48, wherein the mammalian oocyte or fertilized zygote is surrounded by a zona pellucida and wherein the zona pellucida is removed prior to step a).

Claim 51 (previously presented) The method of claim 50, wherein the zona pellucida is removed by a method selected from the group consisting of: (a) treatment with an enzyme or an acidified Tyrodes solution, (b) micromanipulation followed by treatment with a micro filament

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inhibitor and vortexing, and (c) micropipeting in the presence of an microfilament inhibitor with mechanical aspiration of cytoplasm.

Claim 52 (previously presented) The method of claim 51, wherein the enzyme is Pronase.

Claim 53 (previously presented) The method of claim 51, wherein the microfilament inhibitor is cytochalasin B.

Claim 54 (previously presented) The method of claim 48, wherein the mammalian oocyte fertilized zygote, or resulting fragment thereof is enucleated.

Claim 55 (previously presented) The method of claim 54, wherein the mammalian oocyte fertilized zygote, or resulting fragment thereof is enucleated by micromanipulation or centrifugation in an appropriate gradient in the presence of a microfilament inhibitor.

Claim 56 (previously presented) The method of claim 48, wherein the mammalian oocyte is matured *in vivo*.

Claim 57 (previously presented) The method of claim 48, wherein the mammalian oocyte is matured *in vitro*.

Claim 58 (previously presented) The method of claim 48, wherein the mammalian oocyte is selected from the group consisting of: an activated, low maturation promotion factor ("MPF") oocyte; an aged, unactivated, low MPF oocyte; and an unactivated, high MPF, metaphase II oocyte.

Claim 59 (previously presented) The method of claim 58, wherein the mammalian oocyte is an unactivated, high MPF, metaphase II oocyte.

Claim 60 (previously presented) The method of claim 48, wherein the cytoplasmic fragment is from a different species from that of the nuclear donor.

Claim 61 (previously presented) The method of claim 48, wherein the cytoplasmic fragment is from the same species as that of the nuclear donor.

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Claim 62 (previously presented) The method of claim 48, wherein the cytoplasm fragment is prepared from a mammalian oocyte or fertilized zygote taken from a non-human mammalian species.

Claim 63 (previously presented) The method of claim 62, wherein the cytoplasm fragment is prepared from a mammalian oocyte or fertilized zygote taken from a mouse, rat, rabbit, sheep, goat, pig, or cow.

Claim 64 (previously presented) The method of claim 63, wherein the cytoplasm fragment is prepared from a mammalian oocyte or fertilized zygote taken from a cow.

Claim 65 (previously presented) The method of claim 48, wherein the nuclear donor cell is selected from the group consisting of fibroblasts, skin fibroblasts, leukocytes, granulosa cells, cumulus cells, oviductal epithelium, mammary gland cells, fetal fibroblasts, keratinocytes, hepatocytes, respiratory epithelial cells, neuronal cells, CD34+ stem cells, granulocytes, and mononuclear peripheral blood cells.

Claim 66 (previously presented) The method of claim 48, wherein the nuclear donor is a karyoplast.

Claim 67 (previously presented) The method of claim 66, wherein the karyoplast is an interphase cell.

Claim 68 (previously presented) The method of claim 48, further comprising maintaining the pluripotency by placing the cell in a culture media that supports development and proliferation while maintaining the dedifferentiated state.

Claim 69 (previously presented) The method of claim 66, wherein the karyoplast is enriched with mitochondria.

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Claim 70 (previously presented) The method of claim 48, wherein the combining of the cytoplast fragment with the nuclear donor is mediated by electrical fusion, chemical fusion, viruses, liposomes or cell surface proteins.

Claim 71 (previously presented) The method of claim 70, wherein the combining is mediated by electrical fusion.

Claim 72 (previously presented) The method of claim 70, wherein the combining is mediated by polyethylene glycol or high pH-low osmolarity.

Claim 73 (previously presented) The method of claim 48, further comprising an activation step.

Claim 74 (previously presented) The method of claim 73, wherein said activation occurs before the combining step.

Claim 75 (previously presented) The method of claim 73, wherein the activation occurs after the combining step.

Claim 76 (previously presented) The method of claim 73, wherein the activation is mediated by electrical pulse, ionomycin/DMAP, cytochalasin/cyclohexamide, strontium, adenophostin, disintegrin RGD peptide, DDT/thimerosal, ethanol or sperm factor.

Claim 77 (previously presented) The method of claim 48, wherein the nuclear donor is from an embryonic, fetal, or adult cell, or an embryonic, fetal, or adult karyoplast.

Claim 78 (previously presented) The method of claim 48, wherein the nuclear donor is a diploid cell or is taken from a diploid cell.

Claim 79 (previously presented) The method of claim 78, wherein the nuclear donor is from a cell or karyoplast nonsynchronized, synchronized in G0/G1; or by a cell or karyoplast arrested at the G1/S border.

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Claim 80 (previously presented) The method of claim 48, wherein the nuclear donor is optionally matched to the cell cycle stage of the cytoplasm donor.

Claim 81 (previously presented) The method of claim 48, wherein the nuclear donor is from a stem cell, or differentiated or undifferentiated somatic cell.

Claim 82 (previously presented) The method of claim 48, wherein the nuclear donor is from a human, cow, bull, pig, sheep, goat, primate, rodent or lagomorph.

Claim 83 (previously presented) The method of claim 48, wherein the nuclear donor is from a human.

Claim 84 (previously presented) The method of claim 48, wherein the nuclear donor has been genetically modified.

Claim 85 (previously presented) The method of claim 84, wherein the nuclear donor is genetically modified with a gene designed to correct a genetic defect or with a capacity to produce a protein, enzyme, enzyme product, cellular component or a therapeutic agent.

Claim 86 (withdrawn) The method of claim 48, wherein mitochondria of the donor cytoplasm is made replication incompetent.

Claim 87 (withdrawn) The method of claim 86, wherein the cytoplasm fragment is incubated with an inhibitor of mitochondrial DNA replication.

Claim 88 (withdrawn) The method of claim 86, wherein the cytoplasm fragment is incubated with an inhibitor of mitochondrial DNA replication.

Claim 89 (withdrawn) The method of claim 86, wherein the cytoplasm fragment is incubated with EtBr.

Claim 90 (withdrawn) The method of claim 48, wherein the cell is supplemented with mitochondria derived from the same species as the nuclear donor.

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Claim 91 (withdrawn) The method of claim 90, wherein mitochondria is derived from the same animal or individual as the nuclear donor.

Claim 92 (withdrawn) The method of claim 90, wherein the mitochondria supplementation is mediated by fusion of an enucleated cytoplasm with the cell.

Claim 93 (withdrawn) The method of claim 92, wherein the enucleated cytoplasm is derived from platelets.

Claim 94 (withdrawn) The method of claim 48, wherein the nuclear donor is stably transfected with a gene encoding a mitochondrial maintenance factor.

Claim 95 (withdrawn) The method of claim 94, wherein the gene is mtTFA.

Claim 96 (withdrawn) The method of claim 48, wherein the nuclear donor is transiently transfected with a gene encoding a modulator of histone acetylation or a modulator of chromatin structure.

Claim 97 (withdrawn) The method of claim 96, wherein the gene is histone deacetylase.

Claim 98 (previously presented) The method of claim 48, further comprising the step of establishing a population of hybrid cells derived from the hybrid cell.

Claim 99 (canceled)

Claim 100 (canceled)

Claim 101 (withdrawn) The method of claim 98, further comprising the step of culturing the hybrid cell population in the presence of compounds or factors which induce gene transcription, thereby producing an activated hybrid cell population.

Claim 102 (withdrawn) The method of claim 101, wherein the compounds or factors is a reversible inhibitor of histone deacetylase.

Claim 103 (withdrawn) The method of claim 102, wherein the reversible inhibitor of histone deacetylase is butyrate.

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Claim 104 (withdrawn) The method of claim 102, wherein the reversible inhibitor of histone deacetylase is trichostatin A.

Claim 105 (withdrawn) The method of claim 101, further comprising the step of culturing the activated hybrid cell population in a medium that maintains the dedifferentiated state of the activated hybrid cell population and support the development and proliferation of the activated hybrid cell population.

Claim 106 (withdrawn) The method of claim 105, wherein the medium comprises cytokines, L , steel factor, or CGT44.

Claim 107 (withdrawn) The method of claim 105, wherein the medium comprises a feeder layer of mitotically inactivated primary fibroblast cells.

Claim 108 (withdrawn) The method of claim 105, further comprising the step of removing activated hybrid cell population from the medium and culturing the activated hybrid cell population in a second medium which induces differentiation of embryonic stem cells.

Claim 109 (withdrawn) The method of claim 108, wherein the second medium comprises a factor which induces neural pathway differentiation.

Claim 110 (withdrawn) The method of claim 109, wherein the factor is retinoic acid, fibroblast growth factor 2(FGF2), epidermal growth factor (EGF), or platelet-derived growth factor (PDGF).

Claim 111 (withdrawn) The method of claim 108, wherein the second medium comprises c-kit and erythropoietin.

Claim 112 (withdrawn) The method of claim 108, wherein the second medium comprises macrophage colony stimulating factor (M-CSF), interleukin I and interleukin 3.

Claim 113 (withdrawn) The method of claim 108, wherein the second medium comprises retinoic acid, insulin, and tri-iodothyronine.

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Claim 114 (withdrawn) The method of claim 108, wherein the second medium comprises retinoic acid and dibutyl cyclic AMP.

Claim 115 (withdrawn) The method of claim 108, wherein the second medium comprises cells from the pancreatic bud.

Claim 116 (withdrawn) The method of claim 98, further comprising the step of transfecting cells of the hybrid cell population with genes encoding activators or transcription factors.

Claim 117 (withdrawn) The method of claim 98, wherein the cells are transfected with Myo D, PPAR gamma, or C/EBP alpha.

Claim 118 (withdrawn) A method of generating and enriching a population of hybrid cells comprising:

- (a) preparing a population of cytoplasmic fragments stained with a first color;
- (b) preparing a population of nuclear donor cells transfected with a gene that encodes a fluorescent protein, which is capable of fluorescing a second color;
- (c) fusing said population of cytoplasmic fragments and said population of nuclear donor cells, thereby producing a population of products comprising fused products, unfused cytoplasmic fragments, and unfused nuclear donors, wherein the fused products comprise hybrid cells with a normal karyotype and aneuploidy cells;
- (d) sorting the population of products by selecting for fused products and unfused cytoplasmic fragments marked by the first color; and
- (e) further sorting the fused products by selecting for cells with a normal karyotype and marked by the second color.



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Claim 119 (withdrawn) A method of generating and enriching a population of hybrid cells comprising:

- (a) preparing a population of stained cytoplasts stained with a first color;
- (b) preparing a population of nuclear donor cells, wherein the DNA of the nuclear donor cell is stained with a second color;
- (c) fusing said population of cytoplasts and said population of nuclear donor cells, thereby producing a population of products comprising fused products, unfused cytoplasts, and unfused nuclear donors, wherein said fused products comprise hybrid cells with a normal karyotype and aneuploidy cells;
- (d) sorting the population of products by selecting for fused products and unfused cytoplasts marked by the first color; and
- (e) further sorting the fused products by selecting for cells with a normal karyotype marked by the second color.

Claim 120 (previously presented) The method of claim 48 wherein more than 10 cytoplasm fragments are prepared.

Claim 121 (previously presented) The method of claim 48 wherein 10 to 50 cytoplasm fragments are prepared.

Claim 122 (previously presented) The method of claim 48 wherein the hybrid mammalian cell is not totipotent.

Claim 123 (currently amended) A method for reprogramming mammalian cells comprising:

- (a) preparing more than one cytoplasm fragment from a mammalian mammalian metaphase II oocyte or fertilized zygote wherein the amount of cytoplasm in the

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cytoplasm fragment is less than the amount of cytoplasm in the mammalian oocyte or fertilized zygote;

- (b) obtaining a nuclear donor cell or karyoplast taken from a mammal; and
- (c) combining a cytoplasm fragment of step a) with the nuclear donor cell or karyoplast of step b) to produce a reprogrammed mammalian cell; and
- (d) if an oocyte is used in step (a), then activating the oocyte before, during or after step (c).

Claim 124 (currently amended) A method for reprogramming mammalian cells comprising:

- (a) preparing more than one cytoplasm fragment from a mammalian metaphase II oocyte or fertilized zygote wherein the amount of cytoplasm in the cytoplasm fragment is less than the amount of cytoplasm in the mammalian oocyte or fertilized zygote;  
;
- (b) obtaining a nuclear donor cell or karyoplast taken from a mammal; and
- (c) combining cytoplasm fragments of step a) with the nuclear donor cell or karyoplast of step b) to produce a reprogrammed mammalian cell; and
- (d) if an oocyte is used in step (a), then activating the oocyte before, during or after step (c).

Claim 125 (withdrawn) The method of claim 123 or 124 wherein the reprogramming is facilitated by the use of chemical or biologically derived agents known to cause gene reactivation.

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Claim 126 (previously presented) The method of claim 123 or 124, wherein the reprogrammed mammalian cell is a cardiomyocyte.

Claim 127 (currently amended) A method of generating a hybrid mammalian cell comprising:

- (a) preparing more than one cytoplasm fragment from a mammalian metaphase II oocyte or fertilized zygote wherein the amount of cytoplasm in the cytoplasm fragment is less than the amount of cytoplasm in the mammalian oocyte or fertilized zygote;
- (b) obtaining nuclear donor cell or karyoplast taken from a mammal; and
- (c) combining cytoplasm fragments of step a) with the nuclear donor cell or karyoplast of step (b) to produce a hybrid mammalian cell; and
- (d) if an oocyte is used in step (a), then activating the oocyte before, during or after step (c).